Insulin Biosynthesis in HIT Cells

Effects of Glucose, Forskolin, IBMX, and Dexamethasone

GERALD GOLD, RONG-LI QIAN, AND GEROLD M. GRODSKY

Glucose, forskolin, 3-isobutyl-1-methylxanthine (IBMX), and dexamethasone were tested as regulators of proinsulin biosynthesis in HIT T-15 cells, which are glucose-responsive simian virus 40–transformed hamster β-cells. Rate of [^{3}H]leucine incorporation into proinsulin was increased as glucose concentrations were raised from 0 to 20 mM. Biosynthetic rate increases were significant after 48 but not at 4 or 24 h of glucose and were greater for proinsulin than for total extractable proteins. After 48 h, glucose-stimulated proinsulin biosynthesis was unaffected by 10^{-6} M forskolin and/or 3×10^{-5} M IBMX but was specifically and significantly inhibited by 10^{-6} M dexamethasone. Four hours of exposure to dexamethasone had no effect. When cells were incubated for 24 h and then continuously labeled for an additional 24 h, cellular conversion of labeled proinsulin to insulin was increased by glucose, and this increase was reversed or inhibited by 10^{-6} M dexamethasone. Therefore, proinsulin biosynthesis in transformed HIT T-15 cells is regulated in several ways by metabolites and hormones in a manner that compares with biosynthetic regulation in normal β-cells. *Diabetes* 37:160–65, 1988

The HIT T-15 is a clonal line of hamster β-cells transformed by simian virus 40 (1). Like other available lines of transformed rodent β-cells, HIT cells continue in tissue culture to synthesize, store, and secrete insulin (although at lesser rates than normal β-cells) and to respond to various secretory stimulators, e.g., glucagon, cAMP, and potassium depolarization, and to inhibitors, e.g., somatostatin and dexamethasone (1,2). Unlike available cultured lines of transformed rat islet (RIN) cells (3–5), which lose or have poor insulin secretory responses to glucose (6,7), HIT cells continue for many passages to secrete insulin in response to low concentrations of glucose. In these cells, glucose stimulates a single phase of insulin secretion that occurs rapidly, can be potentiated by 3-isobutyl-1-methylxanthine (IBMX), and is concentration dependent but reaches near-maximal rates as it approaches physiologic concentrations of glucose (2). In normal β-cells, glucose is the primary physiologic stimulator of both insulin secretion and insulin biosynthesis (8–10). Because insulin biosynthesis in HIT cells has not been extensively studied, we wanted to determine *1*) whether insulin biosynthesis in this β-cell model is regulated by glucose, nonmetabolizable stimulators, and/or inhibitors; *2*) whether regulation is specific for insulin; and if so, *3*) whether regulation most likely reflects changes in transcriptional (11–13), translational (14,15), or proinsulin-to-insulin processing rates (16).

From the Metabolic Research Unit, University of California, San Francisco, California.
Address correspondence and reprint requests to Dr. Gerald Gold, Room 1157 H.S.W., Metabolic Research Unit, University of California, San Francisco, CA 94143.
Received for publication 19 May 1987 and accepted in revised form 7 July 1987.

MATERIALS AND METHODS

HIT T-15 cells were obtained from A.E. Boyd III (Houston, TX) and R.F. Santerre (Indianapolis, IN) and grown at 37°C in T-75 tissue culture flasks (Falcon, Becton Dickinson, Oxnard, CA) with RPMI-1640 medium containing, unless stated otherwise, 25 mM HEPES, 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (media, sera, and antibiotics were obtained from the Cell Culture Facility at the University of California, San Francisco). Flasks were gassed with 95% air/5% CO_2, and cells were passaged once each week by detachment in saline A containing 0.05% trypsin plus 0.02% EDTA.

Labeling of cells. Experiments were done in T-25 tissue culture flasks (Falcon) containing ~0.5×10^6 HIT T-15 cells (passage 55–66) and 6 ml RPMI-1640 medium. After 3 days, incubation medium was removed and replaced with fresh RPMI-1640 medium containing specified concentrations of glucose, forskolin (Calbiochem, La Jolla, CA), IBMX (Aldrich, Milwaukee, WI), or dexamethasone (Sigma, St. Louis, MO). Medium containing 0, 5, 10, or 20 mM glucose was made from a custom, glucose-free RPMI-1640 and dialyzed calf

serum; 50% sterile glucose was added to achieve the specified glucose concentration. All other media always contained 11.1 mM glucose. Forskolin, IBMX, and dexamethasone were added from ethanol stocks (300- to 1000-fold concentrated); the same volume of ethanol was added to control flasks. Labeling was done by removing incubation medium and replacing with 6 ml fresh labeling medium made from a custom, leucine-free RPMI-1640 medium that contained 63 μCi/ml (final) [^{3}H]leucine (Amersham, Arlington Heights, IL).

Determination of total extractable protein. After a 1- or 24-h period of labeling, incubation medium was discarded, cells were washed with 5.0 ml ice-cold Dulbecco's phosphate-buffered saline, and then extracted for 14–16 h with 5.28 ml ice-cold acid-ethanol [67 ml ethanol + 31 ml water + 1.4 ml concentrated hydrochloric acid + 3 mg bovine serum albumin (fraction V, Sigma)]. All subsequent purification procedures were done at 4°C. Extracts were transferred to a tube containing 50 μl 2 M ammonium acetate and mixed. Total labeled extractable protein was determined by spotting 5 μl on a 7-mm circle of Whatman 3 paper and washing four times at room temperature with 500 ml (total) 10% trichloroacetic acid plus 40 μM leucine. Circles were then air-dried and insoluble radioactivity quantitated in Hydrofluor (National Diagnostics, Manville, NJ) with a Tri-Carb liquid scintillation counter (Packard, Downers Grove, IL).

Purification of insulin and proinsulin. The remainder of each extract was adjusted to pH 8.3 with ammonium hydroxide and, after 30 min, centrifuged 10 min at 1000 × *g*. Supernatants were decanted, readjusted to pH 5.3 with hydrochloric acid, and 10 ml ethanol plus 20 ml ether were added with mixing (17,18). After 3 h on ice, precipitates were collected by centrifugation for 10 min at 1000 × *g*, air-dried, and then redissolved in 1.0 ml buffered detergent [1% (wt/vol) sodium deoxycholate (Sigma) + 1% (wt/vol) Triton X-100 (Sigma) + 0.15 M sodium chloride + 50 μM leucine + 10 mM sodium phosphate, pH 7.4]. Samples were centrifuged 5 min in an Eppendorf centrifuge, and supernatants were collected and incubated overnight at 4°C in tubes containing preformed double-antibody precipitates that had been previously made with 4 μl guinea pig anti-pork insulin serum (Linco, Eureka, MO) plus 100 μl goat anti-guinea pig serum (heavy- and light-chain specific, Cooper, Malvern, PA) plus 336 μl buffered detergent; combined antibodies were incubated 2 h at 37°C and an additional ≥4 h at 4°C before addition of samples (19). After incubation overnight at 4°C, samples were layered over 100 μl 1 M sucrose plus buffered detergent in 1.5-ml Eppendorf tubes and centrifuged 5 min. Supernatants were aspirated and discarded. Pellets were washed three times with a total of 2.1 ml buffered detergent by vigorous vortex mixing followed by centrigugation for 1 min in an Eppendorf centrifuge. Final pellet was resuspended in 0.25 ml 1.0 M acetic acid by trituration and vortex mixing and centrifuged for 5 min in a Eppendorf centrifuge, the supernatant was collected, and radioactivity (proinsulin plus insulin) was quantitated by liquid scintillation counting. Nonspecific binding was assessed by prior addition of 100 μg pork insulin to preformed double-antibody precipitates and represented 9.95 ± 0.77% of total binding in eight typical experiments.

Separation of labeled proinsulin and insulin. Separation and quantitation of labeled proinsulin and insulin was done after 24-h labeling experiments with 1 × 110-cm columns of Bio-Gel P-30 (Bio-Rad, Richmond, CA) and eluting with 3 M acetic acid plus 0.05% bovine serum albumin (Pentex, Miles, Naperville, IL) (18). With HIT cells labeled for 1 h we found that, comparable to short-term–labeled islets (8,18), mature cellular insulin had not yet formed. Therefore, in subsequent experiments with HIT cells labeled for only 1 h, all

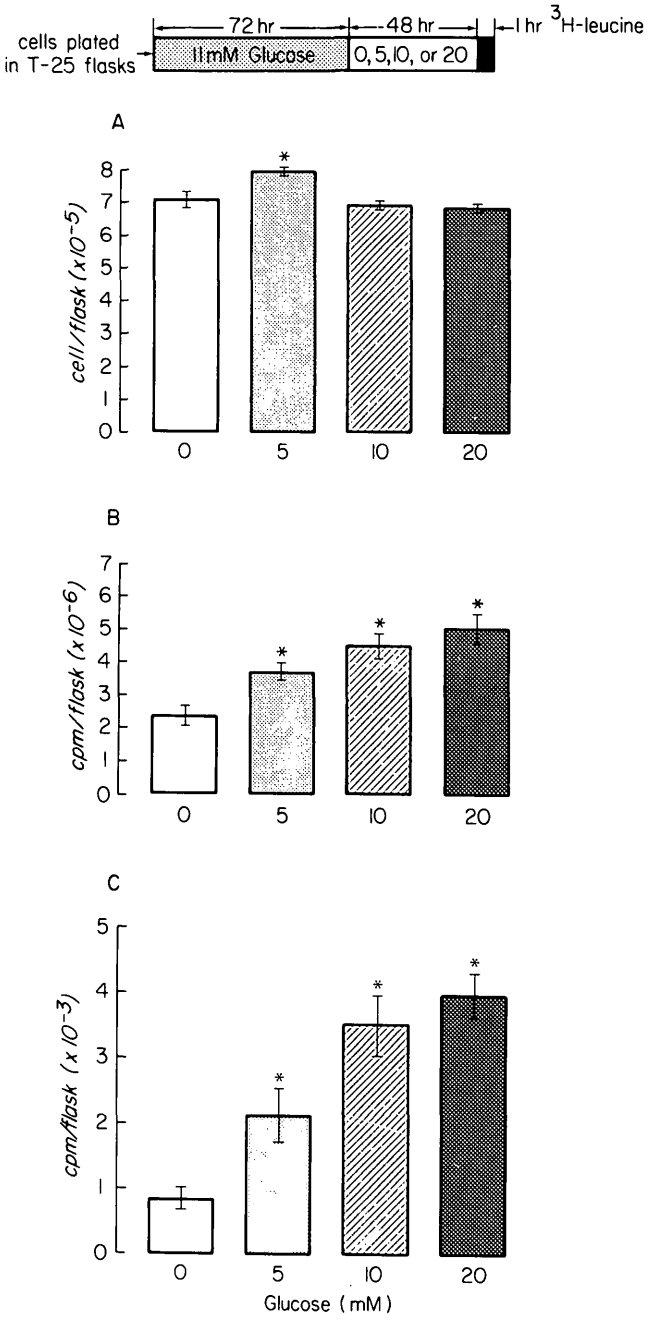

FIG. 1. Effects of 48 h of 0, 5, 10, or 20 mM glucose on cell numbers (*A*, *n* = 10, **P* < .05 vs. 0 mM glucose) and incorporation of [^{3}H]leucine into total extractable protein (*B*, *n* = 12, **P* < .01 vs. 0 mM glucose) and immunopurified proinsulin (*C*, *n* = 12, **P* < .01 vs. 0 mM glucose). Cells were grown for 48 h at 37°C in 6 ml glucose-free RPMI-1640 medium that contained 10% dialyzed calf serum. Medium was supplemented with 2.78 M glucose to attain reported glucose concentrations. After incubation, medium was removed, and labeling was done for 1 h at 37°C with 6 ml leucine-free RPMI-1640 medium containing same concentration of glucose, 10% dialyzed calf serum, and 63 μCi/ml [^{3}H]leucine.

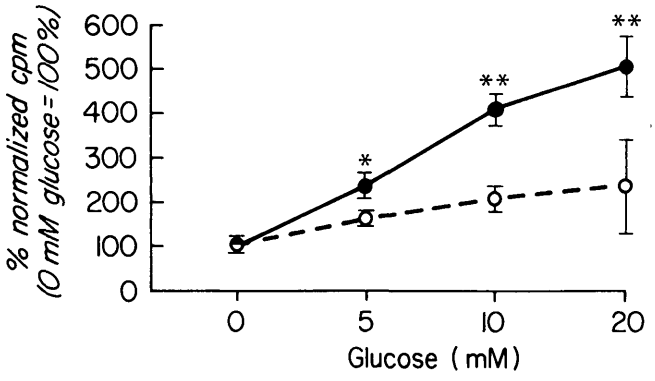

FIG. 2. Effect of glucose concentration on percent increase in [^{3}H]leucine labeling of total extractable proteins and immunopurified proinsulin. Data from same experiments shown in Fig. 1. At 5, 10, and 20 mM glucose, percent normalized counts per minute was significantly greater for immunopurified proinsulin than for total labeled protein. *Solid line*, purified labeled proinsulin; *dashed line*, total labeled protein. *$P < .05$, **$P < .002$.

immunoprecipitated radioactivity was assumed to be proinsulin and, possibly, partially processed intermediates.

Insulin assay. Insulin was measured by radioimmunoassay via guinea pig anti-porcine insulin (Linco) (18) and porcine insulin standards. Hamster insulin standards were not available, and in our radioimmunoassay, incubation media for HIT cells diluted in parallel with porcine but not rat insulin.

Analysis of data. Numbers represent means ± SE for experiments done on different days. Control and experimental conditions were run for each experiment, and differences were assessed by Student's paired *t* test or by one-way analysis of variance for multiple comparisons.

RESULTS

Effects of 48 h of incubation of HIT cells with 0, 5, 10, or 20 mM glucose on cell number, biosynthesis of total protein, and biosynthesis of immunopurified proinsulin are shown in Fig. 1. Although cell number was slightly higher with medium containing 5 mM glucose, there was no progressive pattern of increase to indicate glucose concentration consistently affected cell number within 48 h (Fig. 1*A*). At the end of the incubation period, [^{3}H]leucine incorporation (1 h) into total extractable protein was consistently and progressively increased with increasing glucose concentrations (Fig. 1*B*). Biosynthetic rate of immunopurified proinsulin also was significantly and progressively augmented with increasing glucose concentrations (approaching a maximum at 10 mM glucose; Fig. 1*C*). At 20 mM glucose, labeled proinsulin represented 0.079% of total labeled protein, whereas at 0 mM glucose, it represented only 0.036%. Thus, when glucose concentration was raised from 0 to 20 mM, there was a fivefold increase of proinsulin biosynthesis in HIT cells representing the sum of a greater than twofold proinsulin-specific increase plus a twofold nonspecific increase of total protein biosynthesis (Fig. 2).

Effects of 0 or 20 mM glucose on rate of proinsulin biosynthesis (1-h labeling) were also tested after 4- and 24-h incubations (Fig. 3). After 4 h, labeling of either total extractable protein or proinsulin was unaffected by glucose concentration in media. After 24 h, labeling of both total extractable protein and proinsulin were comparably, but not significantly, elevated by 20 mM glucose. Thus, although insulin secretion in perifused HIT cells has been reported to respond within minutes to increasing glucose concentrations (2), 48 h were required to demonstrate significant stimulation of proinsulin biosynthesis under conditions used here.

Forskolin and IBMX are two compounds that increase cellular levels of cAMP (2,20). Both IBMX (2) and forskolin (20,21) have been reported to rapidly potentiate glucose-stimulated insulin secretion in HIT cells, and we found in three 1-h incubations that 25 μM forskolin significantly ($P < .001$) increased fractional release of insulin with glucose-stimulated (20 mM) cells from 10.6 ± 2.4 to 25.3 ± 7.5%/h. Effects of forskolin and IBMX were determined after 48 h with either or both added to media containing 11.1 mM glucose. Biosynthesis of total extractable protein and of proinsulin was unaffected by incubation with these compounds alone or in combination (Fig. 4). Therefore, as with normal β-cells, regulation of proinsulin biosynthesis and regulation of insulin secretion are separable (8,10).

Biosynthetic effects of dexamethasone were tested at a pharmacologic concentration (10^{-6} M). Dexamethasone is known to affect insulin secretion in isolated islets (22–25),

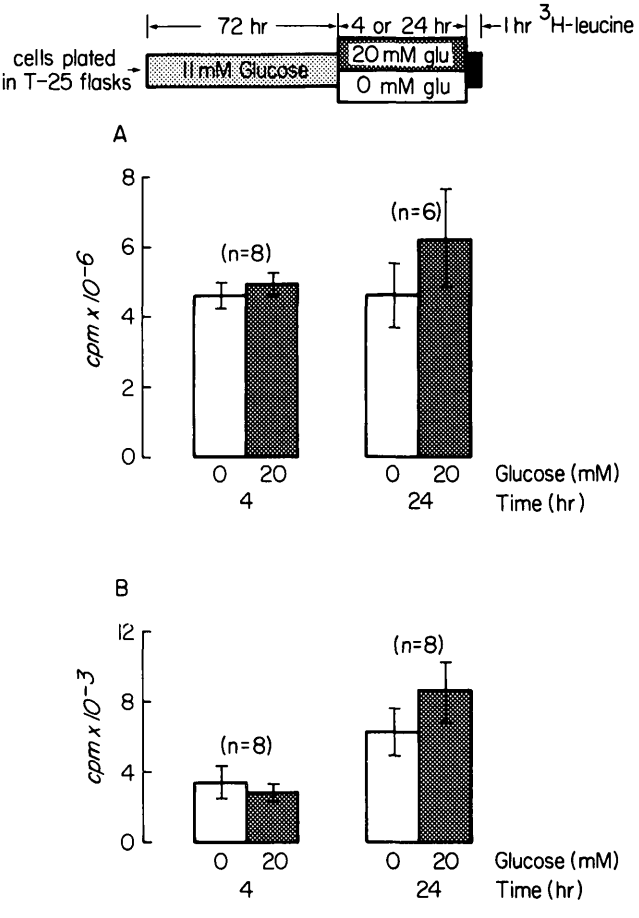

FIG. 3. Effect of 4 or 24 h of 0 or 20 mM glucose on [^{3}H]leucine labeling of total extractable proteins (*A*) and of immunopurified proinsulin (*B*). Cells were grown for either 4 or 24 h at 37°C in 6 ml glucose-free RPMI-1640 medium that contained 10% dialyzed calf serum. Medium was supplemented with 2.78 M glucose to attain reported glucose concentrations. After incubation medium was removed, labeling was done for 1 h at 37°C with 6 ml leucine-free RPMI-1640 medium containing 63 μCi/ml [^{3}H]leucine as described in Fig. 1.

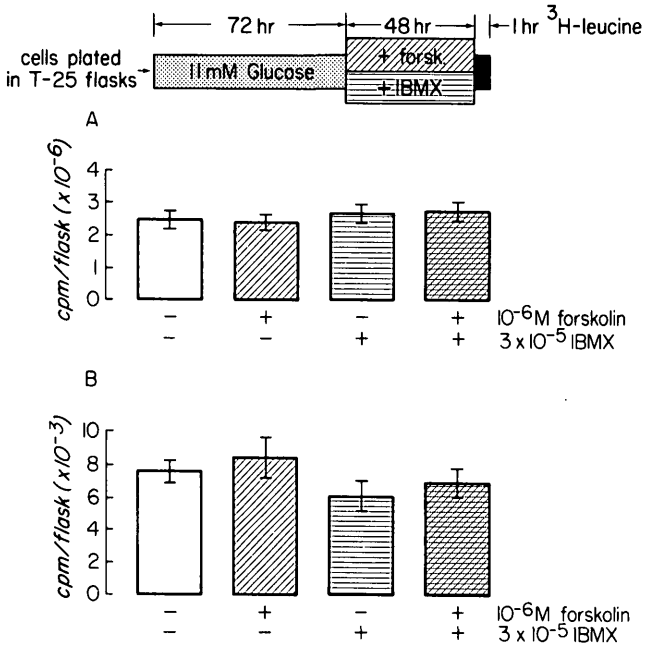

FIG. 4. Effects of 48 h of 10^{-6} M forskolin and 3 × 10^{-5} M 3-isobutyl-1-methylxanthine (IBMX) on incorporation of [³H]leucine into total extractable protein (*A*, *n* = 10) and immunopurified proinsulin (*B*, *n* = 10). Cells were grown for 48 h at 37°C in 6 ml RPMI-1640 medium that contained 10% fetal bovine serum. Medium was supplemented with ethanolic stocks of 10^{-3} M forskolin and 10^{-2} M IBMX to attain reported concentrations; control flasks also contained 0.3% (vol/vol) ethanol. After incubation medium was removed, labeling was done for 1 h at 37°C in 6 ml leucine-free RPMI-1640 medium containing same concentration of forskolin, IBMX or ethanol, 10% fetal bovine serum, and 63 μCi/ml [³H]leucine.

and 10^{-6} M dexamethasone severely inhibits insulin secretion in HIT cells (1). After 4 h of dexamethasone treatment, there was no effect on labeling of either total extractable protein or proinsulin (Fig. 5). After 48 h, 10^{-6} M dexamethasone modestly lowered total protein synthesis by 27% (not significantly different from controls); however, it significantly ($P < .001$) decreased proinsulin synthesis by >80% (Fig. 6). Thus, 10^{-6} M dexamethasone required 48 h to produce a small nonspecific and a much larger proinsulin-specific inhibition of glucose-stimulated protein biosynthesis.

In a separate series of studies, HIT cells were incubated for 24 h and then labeled with [³H]leucine for an additional 24 h in media containing 0, 5, 10, and 20 mM glucose. Percent intact labeled cellular proinsulin (including partially processed 9000-*M*, intermediates) was slightly (not significantly) decreased from 26% at 0 mM to 18% with 5 mM glucose and significantly ($P < .05$) decreased to <14% at 10 and 20 mM glucose (Fig. 7). Cells were also incubated for 24 h and then labeled with [³H]leucine for an additional 24 h in RPMI-1640 media containing 10^{-6} M dexamethasone. In these experiments, 20% of the labeled cellular hormone was intact proinsulin in control incubations containing 11.1 mM glucose, whereas this percentage was significantly ($P < .001$) increased to >35% when 10^{-6} M dexamethasone was present (Fig. 8).

DISCUSSION

There are both general similarities and important differences between normal rodent islets and HIT cells. Rodent islets are heterogeneous, difficult to isolate in quantity, have a low mitotic index, but demonstrate insulin production representing from 10 to as much as 50% of total protein biosynthesis (8). Although normal β-cells can now be enriched by cell sorting of disaggregated islets, most studies of insulin biosynthesis have used fresh or cultured intact islets that contain cells that also produce glucagon, somatostatin, and pancreatic polypeptide. Transformed HIT T-15 cells, on the other hand, are homogeneous β-cells that are plentiful, have a high mitotic index, but demonstrate insulin production representing only ≤0.1% of total protein biosynthesis. We and others have been unable to measure immunoreactive glucagon or somatostatin in HIT T-15 cells (1; unpublished observation). However, functional heterogeneity still exists within clonal cell lines, and heterogeneity among HIT cells should be noted as a possible source of proinsulin-specific effects.

In normal islets, all steps of insulin biosynthesis are specifically regulated by glucose. Glucose slowly increases level of preproinsulin mRNA by enhancing rate of transcription (12) and by stabilizing existing preproinsulin mRNA (13). It also increases overall rates of proinsulin production within 30 min, a period in which levels of preproinsulin mRNA are constant (14) and rates of translational initiation (11) and elongation (15) are enhanced. In HIT cells, Hammonds et

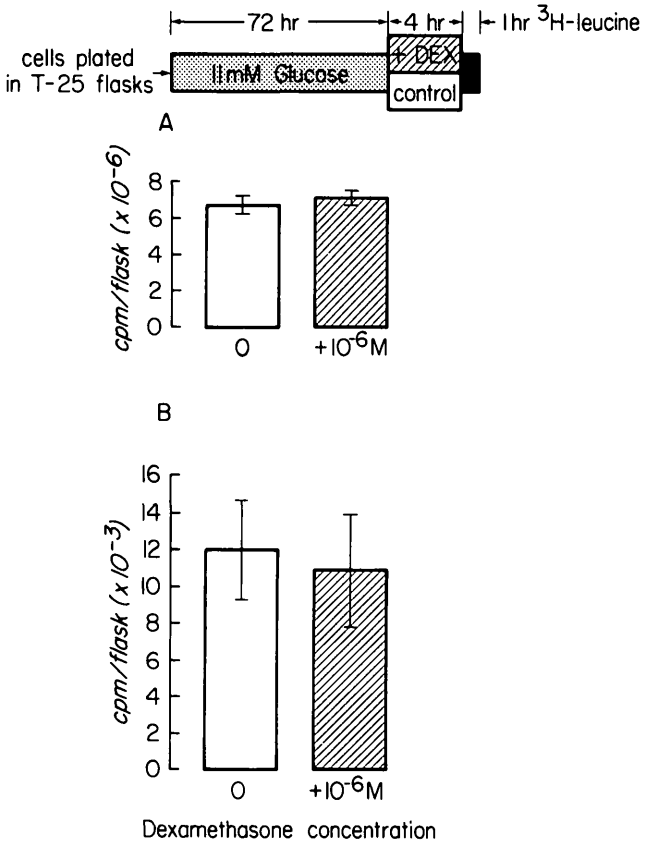

FIG. 5. Effects of 4 h of 10^{-6} M dexamethasone on [³H]leucine labeling of total extractable proteins (*A*, *n* = 8) and immunopurified proinsulin (*B*, *n* = 8). Cells were grown for 4 h at 37°C in 6 ml RPMI-1640 medium that contained 10% fetal bovine serum and was supplemented with ethanolic stock of 10^{-3} M dexamethasone to attain reported concentration; control flasks contained 0.1% (vol/vol) ethanol. After incubation medium was removed, labeling was done for 1 h at 37°C in 6 ml leucine-free RPMI-1640 medium containing same concentration of dexamethasone, 10% fetal bovine serum, and 63 μCi/ml [³H]leucine.

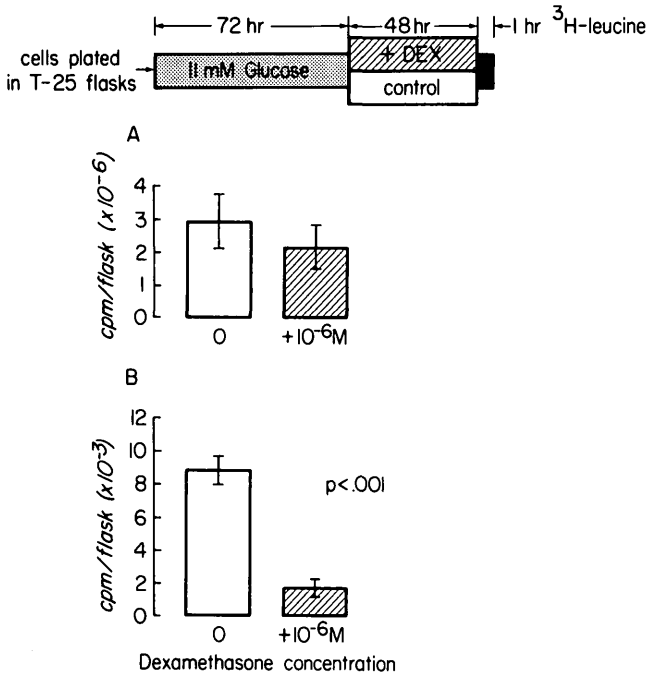

FIG. 6. Effects of 48 h of 10^{-6} M dexamethasone on [^{3}H]leucine labeling of total extractable proteins (*A*, *n* = 12) and immunopurified proinsulin (*B*, *n* = 12). Cells were grown for 48 h at 37°C in 6 ml RPMI-1640 medium that contained 10% fetal bovine serum. Medium was supplemented with ethanolic stock of 10^{-3} M dexamethasone to attain reported concentrations; control flasks contained 0.10% (vol/vol) ethanol. After incubation medium was removed, labeling was done for 1 h at 37°C in 6 ml leucine-free RPMI-1640 medium containing 63 μCi/ml [^{3}H]leucine as described in Fig. 5.

al. (21) have reported that glucose significantly elevates levels of preproinsulin mRNA within 4 h. We found here that glucose required 48 h to produce significant increases in rate of proinsulin labeling, which is consistent with a significant biosynthetic effect of glucose occurring at the level of preproinsulin mRNA synthesis or stabilization in HIT cells (21). To a lesser extent, glucose also nonspecifically enhanced rates of total protein biosynthesis. Like islets, biosynthetic regulation in HIT cells did not require significant cellular division. Also, like islets (10), relatively slow regulation of glucose-stimulated insulin biosynthesis in HIT cells was not constitutively linked to relatively rapid regulation of insulin secretion (2). In islets, ~7 mM glucose produces half-maximal biosynthetic stimulation (8–10); effects were comparable in these HIT T-15 cells.

Forskolin and IBMX had essentially no effect on glucose-stimulated rates of proinsulin labeling with HIT cells. In islets, enhanced levels of cAMP also have been found to have little if any effect on rate of insulin biosynthesis. However, there may be a minor role for cAMP in regulation of insulin biosynthesis, because in HIT cells, forskolin, but not theophylline (a phosphodiesterase inhibitor comparable to IBMX), enhanced levels of preproinsulin mRNA (21); in RIN cells, cAMP has been implicated as part of the glucose-initiated signal for enhanced insulin mRNA transcription (12). Forskolin, theophylline (20,21), and IBMX (2) have all been reported to potentiate glucose-stimulated insulin release, providing additional evidence that there are conditions for which regulation of insulin biosynthesis and secretion are separable in HIT cells.

In isolated rodent islets, glucocorticoids have been reported to have essentially no physiologic effect or to inhibit insulin biosynthesis and secretion, particularly at low glucose concentrations (22–25). Glucocorticoids (via their receptors) alter transcriptional rates of specific genes and bind tRNA species required for general protein synthesis (26).

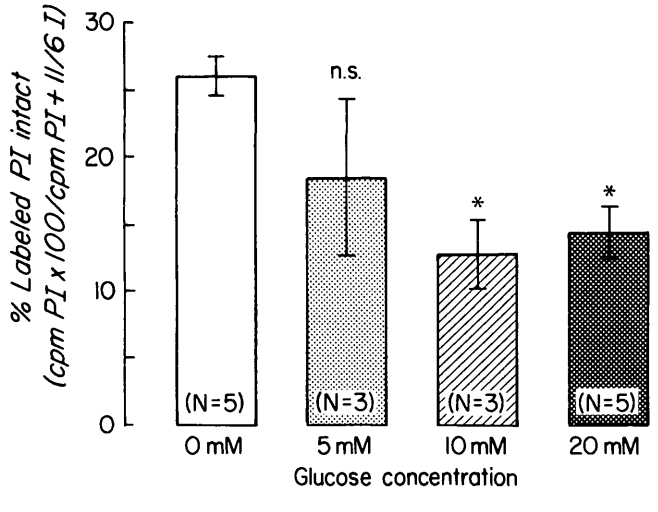

FIG. 7. Effect of glucose concentration on cellular conversion of labeled proinsulin (PI) to insulin (I). Cells were grown for 24 h at 37°C in 6 ml glucose-free RPMI-1640 medium that contained 10% dialyzed calf serum. Medium was supplemented with 2.78 M glucose to attain reported glucose concentrations. After incubation medium was removed, labeling was done for 24 h with 6 ml leucine-free RPMI-1640 medium containing same concentration of glucose, 10% dialyzed calf serum, and 63 μCi/ml [^{3}H]leucine. *P < .05 vs. 0 mM glucose.

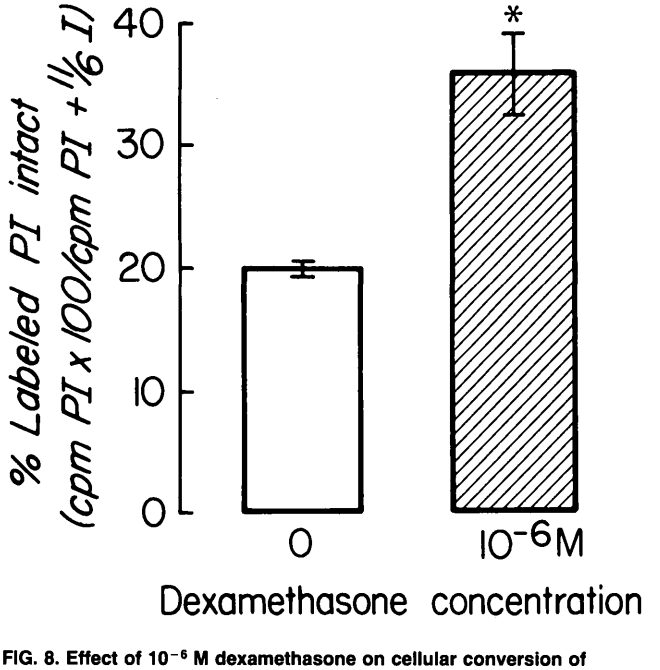

FIG. 8. Effect of 10^{-6} M dexamethasone on cellular conversion of labeled proinsulin (PI) to insulin (I). Cells were grown for 24 h at 37°C in 6 ml RPMI-1640 medium that contained 10% fetal bovine serum. Medium was supplemented with ethanolic stock of 10^{-3} M dexamethasone to attain reported concentration; control flasks contained 0.1% (vol/vol) ethanol. After incubation medium was removed, labeling was done for 24 h at 37°C in 6 ml leucine-free RPMI-1640 medium containing same concentration of dexamethasone, 10% fetal bovine serum, and 63 μCi/ml [^{3}H]leucine. *P < .001, n = 6.

Either mechanism could explain the observed inhibition of proinsulin biosynthesis in HIT cells. Inhibition was proinsulin specific compared with general protein synthesis and reached levels of significance at 48 but not 4 h. As previously explained, this relatively slow biosynthetic effect of dexamethasone favors the possibility that changing levels of preproinsulin mRNA may be responsible for regulation. However, actual measurements of concentrations of preproinsulin mRNA are required to distinguish between transcriptional versus translational regulation.

In normal islets, mature insulin first appears 15–30 min after proinsulin biosynthesis; subsequent conversion to insulin follows pseudo–first-order kinetics (half-life 20–60 min; 8,18,25) and occurs in coated secretory granules (27). In HIT cells, mature insulin was not detectable after a 1-h labeling, suggesting a slower-than-normal conversion or transport from site of synthesis in rough endoplasmic reticulum to secretory granules. Although mature hormone production was slow, HIT cells produced an apparently authentic 6000-M_r hamster insulin that also cochromatographed with pancreatic Syrian hamster insulin on a reverse-phase high-performance liquid chromatograph that can separate several closely related species of insulin (28).

Rate of conversion of proinsulin to insulin is regulated in normal rat islets (16): when islets are first exposed to elevated concentrations of glucose and then labeled, subsequent conversion of labeled proinsulin to insulin is accelerated (16); glucose concentration after labeling has no effect (8,29,30). In HIT cells, elevated glucose concentrations decreased the ratio of proinsulin to total cellular proinsulin plus insulin, suggesting an increased conversion of labeled cellular proinsulin to insulin. Dexamethasone blocked or reversed this effect. These findings indicate that hormone conversion is another site of regulation in HIT cells; however, with a 24-h labeling, selective degradation of insulin or preferential secretion of newly synthesized insulin or proinsulin (possibly by a constitutive pathway; 31) could also account for these results.

In summary, rates of insulin biosynthesis in HIT cells were increased by 5–20 mM glucose, synthesis was not additionally increased by compounds known to elevate cAMP, and synthesis was decreased by 10^{-6} M dexamethasone. Regulation required 48 h to produce significant changes, affected biosynthesis of proinsulin to a greater extent than that of total extractable proteins, and affected extent of conversion of proinsulin to insulin in cellular storage. Whether information for these forms of biosynthetic regulation is encoded within the insulin gene, is intrinsic for secretory proteins in β-cells, or is a combination of both is a question amenable to study in these cultured HIT cells (16).

ACKNOWLEDGMENTS

This work was supported by NIH Grant DK-01410, the Diabetes Research and Education Foundation, and Mr. and Mrs. Henry Snyder in memory of Sonia Goldstein.

REFERENCES

1. Santerre RF, Cook RA, Crisel RMD, Sharp JD, Schmidt RJ, Williams DC, Wilson CP: Insulin synthesis in a clonal line of simian virus 40-transformed hamster pancreatic beta cells. *Proc Natl Acad Sci USA* 78:4339–43, 1981
2. Hill RS, Boyd AE III: Perifusion of a clonal cell line of simian virus 40-transformed beta cells: insulin secretory dynamics in response to glucose, 3-isobutyl-1-methylxanthine and potassium. *Diabetes* 34:115–20, 1985
3. Gylfe E, Andersson T, Rorsman P, Abrahamsson H, Arkhammer P, Hellman P, Hellman B, Oie HK, Gazdar AF: Depolarization-independent net uptake of calcium into clonal insulin-releasing cells exposed to glucose. *Biosci Rep* 3:927–37, 1983
4. Halban PA, Praz GA, Wollheim CB: Abnormal glucose metabolism accompanies failure of glucose to stimulate insulin release from a rat pancreatic cell line (RIN m5F). *Biochem J* 212:439–43, 1983
5. Bhathena SJ, Awoke S, Voyles NR, Wilkins SD, Recant L, Oie HK, Gazdar AF: Insulin, glucagon and somatostatin secretion by cultured rat islet cell tumor and its clones. *Proc Soc Exp Biol Med* 175:35–38, 1984
6. Praz GA, Halban PA, Wollheim CB, Blondel B, Strauss AJ, Renold AE: Regulation of immunoreactive-insulin release from a rat cell line (RIN m5F). *Biochem J* 210:345–52, 1983
7. Giroix MH, Sener A, Dufrane SP, Malaisse-Lagae F, Malaisse WJ: Glucose metabolism in insulin-producing tumoral cells. *Arch Biochem Biophys* 241:561–70, 1985
8. Steiner DF, Kemmler W, Clark JL, Oyer PE, Rubenstein AH: The biosynthesis of insulin. In *Handbook of Physiology. Endocrinology*. Sect. 7, vol. I. Steiner DF, Freinkel N, Eds. Bethesda, MD, Am. Physiol. Soc., 1974, p. 175–98
9. Ashcroft SJH: Glucoreceptor mechanisms and the control of insulin release and biosynthesis. *Diabetologia* 18:5–15, 1980
10. Gold G, Grodsky GM: The secretory process of B cells of the pancreas. In *Cell Biology of the Secretory Process*. Cantin M, Ed. Montreal, Canada, Karger, 1984, p. 359–88
11. Permutt MA, Kipnis DM: Insulin biosynthesis. I. On the mechanism of glucose stimulation. *J Biol Chem* 274:1194–99, 1972
12. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF: Control of insulin gene expression in pancreatic B-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J Biol Chem* 260:13585–89, 1985
13. Steiner DF, Chan SJ, Welsh JM, Kwok SCM: Structure and evolution of the insulin gene. *Annu Rev Genet* 19:463–84, 1985
14. Itoh N, Okamoto H: Translational control of proinsulin synthesis by glucose. *Nature* (Lond) 283:100–102, 1980
15. Welsh M, Scherberg N, Gilmore R, Steiner DF: Translational control of insulin biosynthesis: evidence for regulation of elongation, initiation and signal-recognition-particle-mediated translational arrest by glucose. *Biochem J* 235:459–67, 1986
16. Nagamatsu S, Bolaffi JL, Grodsky GM: Direct effects of glucose on proinsulin synthesis and processing during desensitization. *Endocrinology* 120:1225–31, 1987
17. Davoren PR: The isolation of insulin from a single cat pancreas. *Biochim Biophys Acta* 63:150–53, 1962
18. Gold G, Landahl HD, Gishizky ML, Grodsky GM: Heterogeneity and compartmental properties of insulin storage and secretion in rat islets. *J Clin Invest* 69:554–63, 1982
19. Lomedico PT, Saunders CF: Preparation of pancreatic mRNA: cell-free translation of an insulin-immunoreactive polypeptide. *Nucleic Acids Res* 3:381–91, 1976
20. Ashcroft SJH, Hammonds P, Harrison DE: Insulin secretory responses of clonal cell line of simian virus 40-transformed B cells. *Diabetologia* 29:727–33, 1986
21. Hammonds P, Schofield PN, Ashcroft SJH: Glucose regulates preproinsulin messenger RNA levels in a clonal cell line of simian virus 40-transformed B cells. *FEBS Lett* 213:149–54, 1987
22. Brunstedt J, Nielsen JH: Direct long-term effects of hydrocortisone on insulin and glucagon release from mouse pancreatic islets in tissue culture. *Acta Endocrinol* 96:498–504, 1981
23. Billaudel B, Mathias PCF, Sutter BCJ, Malaisse WJ: Inhibition by corticosterone on calcium inflow and insulin release in rat pancreatic islets. *J Endocrinol* 100:227–33, 1984
24. Khatin MS, Mahmood HA, Gumaa KA, Hellerstrom C: Effect of steroid hormones on DNA synthesis and insulin production of isolated foetal rat pancreatic islets in tissue culture. *Med Biol* 62:210–13, 1984
25. Pierluissi J, Navas FO, Ashcroft SJH: Effect of adrenal steroids on insulin release from cultured islets of Langerhans. *Diabetologia* 29:119–21, 1986
26. Ali M, Vedeckis WV: The glucocorticoid receptor protein binds to transfer RNA. *Science* 235:467–70, 1987
27. Orci L, Ravozzola M, Amherdt M, Madsen O, Vassalli JD, Perrelet A: Direct identification of prohormone conversion site in insulin-secreting cells. *Cell* 42:671–81, 1985
28. Gold G, Walker MD, Edwards DL, Grodsky GM: Effects of glucose and dexamethasone (DEX) on biosynthesis of endogenous and exogenous insulin (Ins) in HIT cells transfected with rat Ins II gene (Abstract). *Diabetes* 36 (Suppl. 1):95A, 1987
29. Steiner DF: Proinsulin and the biosynthesis of insulin. *N Engl J Med* 280:1106–13, 1969
30. Baird N: In vitro regulation of proinsulin synthesis in isolated rat islets (Abstract). *Diabetes* 20 (Suppl. 1):332, 1971
31. Moore HP, Gumbiner B, Kelly RB: Chloroquine diverts ACTH from a regulated to a constitutive secretory pathway in AtT-20 cells. *Nature* (Lond) 302:434–36, 1983